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Low serum bilirubin concentration is a novel risk factor for the development of albuminuria in patients with type 2 diabetes

Hiroshi Okada[a], Michiaki Fukui[a,*], Muhei Tanaka[a], Shinobu Matsumoto[a], Kanae Kobayashi[a], Hiroya Iwase[a], Kiichiro Tomiyasu[b], Koji Nakano[c], Goji Hasegawa[a], Naoto Nakamura[a]

[a] Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan
[b] Department of Cardiology, Kyoto Yamashiro General Medical Center, Japan
[c] Department of Endocrinology and Metabolism, Kyoto Yamashiro General Medical Center, Japan

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ABSTRACT

Objective. Bilirubin has been recognized as an important endogeneous antioxidant. Previous studies reported that bilirubin could prevent atherosclerosis. The aim of this study was to investigate if serum bilirubin concentration could be a predictor for the development of albuminuria in patients with type 2 diabetes.

Materials and Methods. We measured serum bilirubin in 320 consecutive patients with normoalbuminuria. We performed follow-up study to assess the development of albuminuria, mean interval of which was 3.2 ± 0.9 years. Cox proportional hazards regression was used to examine the relationship between serum bilirubin concentration and the development of albuminuria.

Results. During follow-up duration, 43 patients have developed albuminuria. In multivariate analysis, after adjusting for comprehensive risk factors, the risk of developing albuminuria was higher in the lowest quartile of serum bilirubin concentrations than that in the highest quartile of serum bilirubin concentrations (Hazard ratio, 5.76; 95% CI, 1.65 to 24.93).

Conclusions. Low serum bilirubin concentration could be a novel risk factor for the development of albuminuria in patients with type 2 diabetes.

1. Introduction

Cardiovascular disease (CVD) has become the most common cause of mortality and morbidity in patients with type 2 diabetes, and several risk factors including smoking, hypertension and dyslipidemia have been shown to accelerate the progression of CVD [1,2]. Albuminuria, the earliest manifestation of nephropathy, is a marker of increased cardiovascular mortality and the progression of CVD [3,4]. The finding of microalbuminuria is an indication for screening for possible

Abbreviations: CVD, cardio vascular disease; UAE, urinary albumin excretion; BMI, body mass index; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; ANOVA, analysis of variance; SBP, systolic blood pressure; CI, confidence interval; CCB, calcium-channel blockers; LDL, low-density lipoprotein; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1.

* *Corresponding author.* Tel.: +81 75 251 5505; fax: +81 75 252 3721.
E-mail address: sayarinapm@hotmail.com (M. Fukui).

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vascular disease and aggressive intervention to reduce all cardiovascular risk factors [5].

Bilirubin has been shown to be an effective endogenous antioxidant [6–9], to suppress the oxidation of lipids and lipoproteins, especially low-density lipoprotein cholesterol [10], and to be directly related to the total serum antioxidant capacity in humans [9]. Because of its antioxidant, bilirubin could act against plaque formation and subsequent atherosclerosis [11]. Previous studies reported that serum bilirubin has been consistently shown to be inversely related to CVD [12–14]. Inoguchi et al. [15] reported lower prevalence of vascular complications such as coronary artery disease, cerebrovascular disease, retinopathy, or macroalbuminuria in patients with diabetes and Gilbert syndrome, a congenital hyperbilirubinemia. Moreover, recent studies showed serum bilirubin to be associated with CVD-related factors such as hypertension [16,17], abnormal glucose tolerance [18], body mass index [16,19,20], metabolic syndrome [19,21], and coronary artery calcification scores [22]. Serum bilirubin has also been inversely related to the severity of nonalcoholic fatty liver disease, a condition closely related to type 2 diabetes and CVD [23]. And, we previously reported that the serum bilirubin concentration is associated with urinary albumin excretion (UAE) in patients with type 2 diabetes in a cross-sectional study [24]. However, a relationship between serum bilirubin concentration and the development of albuminuria in patients with type 2 diabetes has not been investigated. Therefore, we evaluated the relationship between serum bilirubin concentration and the development of albuminuria in patients with type 2 diabetes.

2. Methods

2.1. Patients and study design

We performed a retrospective cohort study with 320 participants recruited from the outpatient clinic at the Kyoto Prefectural University of Medicine and Kyoto Yamashiro General Medical Center from April, 2006 to June, 2013. Type 2 diabetes was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [25]. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Patients were classified as never smokers, previous smokers, or current smokers according to a self-administered questionnaire. Patients with albuminuria, defined as UAE equal to or more than 30 mg/g creatinine, those with hematuria, bacteriuria, advanced renal dysfunction (serum creatinine more than 2.0 mg/dL), liver cirrhosis, malignancy or hematologic disease, and those with major cardiovascular event during a follow up were excluded from this study. Moreover, we excluded patients (n = 42) who were newly prescribed angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARB) for the first time during follow up period of this study because antihypertensive drugs such as ARB could delay the development of albuminuria in patients with diabetes [5]. We then calculated the hazard ratio for development of albuminuria in patients with normoalbuminuria. This study was approval by the local Research Ethics Committee and was conducted in accordance with Declaration of Helsinki, and informed consent was obtained from all participants.

2.2. Data collection

All data had been retrieved from a database. Overnight fasting blood samples were taken in the morning at baseline. Total serum bilirubin concentrations were measured by an enzymatic method with bilirubin oxidase on an automatic analyzer (Hitachi 7600; Hitachi High-Tchnologies, Tokyo, Japan). Serum total cholesterol and triglyceride concentrations were assessed using standard enzymatic methods. Hemoglobin A1c was assayed using high-performance liquid chromatography and expressed with the unit defined by the National Glycohemoglobin Standardization Program. Urinary albumin and creatinine concentration was determined in an early morning spot urine. UAE was measured with an immunoturbidimetric assay. A mean value for UAE was determined from 3 urine collections. As a follow-up study, we collected urine samples for calculation of UAE at the end of the follow-up interval. The development of albuminuria was defined as UAE equal to or more than 30 mg/g Cr [5].

2.3. Statistical analysis

Means and frequencies of potential confounding variables were calculated. The participants were categorized according to the quartiles of their serum bilirubin concentrations in order to examine the association between patient characteristics at baseline and serum bilirubin concentration: < 0.58, 0.58–0.72,

Table 1 – Characteristics of patients.

n	320
Age (y)	64.0 (10.1)
Sex (male/female)	197/123
Duration of diabetes (y)	12.6 (10.4)
Body mass index (kg/m^2)	23.4 (4.2)
Average systolic blood pressure (mmHg)	128.8 (12.9)
Bilirubin (mg/dL)	0.77 (0.28)
Hemoglobin A1c (%)	7.4 (1.3)
Total cholesterol (mmol/L)	5.0 (0.8)
Triglycerides (mmol/L)	1.4 (0.8)
Aspartate aminotransferase (IU/L)	23.6 (15.7)
Alkaline phosphatase (IU/L)	23.9 (13.7)
Uric acid (μmol/L)	302.5 (86.8)
Creatinine (μmol/L)	65.2 (21.1)
Smoking (never/previous/current)	210/69/41
Retinopathy (NDR/SDR/PDR)	245/36/39
Urinary albumin excretion (mg/g creatinine)	12.8 (7.5)
History of cardiovascular disease (–/+)	269/51
Antidiabetic treatment (insulin/OHA/diet only)	92/197/31
Antihypertensive drug (calcium channel blockers/ACE inhibitor/ARB/diuretic drug/alpha blockers/beta blockers)	84/14/118/23/14/15
Statin (–/+)	179/141

Data are expressed as mean (SD) or absolute number. NDR, no diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; OHA, oral hypoglycemic agent; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers.